EVALUATION FOR ANTI-FUNGAL ACTIVITY

OF

NEOLAMARCKLA CDAMBA LEAF EXTRACT

(This report presented in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy)

A project submitted by:

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APPROVAL

This is notified that a Project report, A few innovative preparations of herbs originated functional cosmetics**,** submitted by 153-29-860 to the Department of Pharmacy, Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy and approved as to its style and contents.

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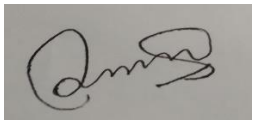

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DECLARATION

I hereby declare that, this project report is done by me under the supervision of **Dr. Mohammed Shafikur Rahman, Assistant Professor,** Department of Pharmacy, Daffodil International University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy. I am declaring that this Project is my original work. I also declare that neither this project nor any part thereof has been submitted elsewhere for the award of Bachelor or any degree.

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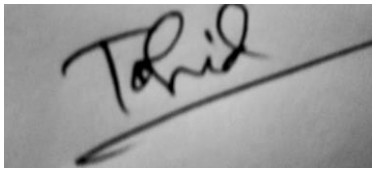

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Md.Tohidul Islam

DECEMBER 2019

Dedicated to

DECLARATION

I hereby declare that this thesis entitled "Evaluation of antifungal activity of Neolamarckia cadamba LEAF extract" is the result of my own research work and effort. It has not been submitted anywhere for any award. Where other sources of information have been used, they have been acknowledged.

Signature

Name : Md. Tohidul Islam

Date : 01-12-2019

LIST OF ABBREVIATIONS

A·flavus	*Aspergillus flavus*
A. fumigatus	*Aspergillus fumigatus*
A. niger	*Aspergillu sniger*
C. albicans	*Candida albicans*
F. solani	*Fusarium solani*
N. cadamba	*Neolamarckia cadamba*
DMSO	Dimethyl sulfoxide
MIC	Minimum Inhibitory Concentration
PDA	Potato Dextrose Agar
SD	Standard deviation
%	Percentage
~l	Microlitre
Cm	*Centimetre*
Mm	Mililitre
mg/mL	Miligram per mililit

Evaluation of Antifungal Activity of Neolamarckia cadamba Leaf Extract

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ABSTRACT

This project focused on antifungal activity of Neolamarckia cadamba leaf extract in order to evaluate the effect of aqueous and ethanol extracts from white kelampayan leaf against the most important phytopathogenic fungi, Aspergillus flavus, Aspergillus niger and Fusarium solani by poisoned food technique. Different concentrations (5, 15, 25, 50 and 100 mglml) prepared from these extracts was inhibited the growth of the experimental fungi and the effect inconsistent with concentration. In the solvent extracts tested, aqueous and ethanol leaf extract gave the highest percentage of inhibition against Aspergillus niger followed by Fusarium solani and Aspergillus flavus. However, aqueous leaf extract have higher percentage of growth inhibition with 31-45% compared to ethanol leaf extract for all experimental fungi. The different concentration of aqueous and ethanol leaf extract have showed inconsistent percentage of growth of inhibition for all experimental fungi which it was slightly not effective in antifungal activity of N. cadamba.

Keywords: Neolamarckia cadamba, Antifungal activity, Aqueous extract, Ethanol extract
Poisoned food technique

1.0 INTRODUCTION

Many of plants are natural sources and used asactive ingredient for medical purpose or compounds for production of new drugs. Medicinal plants are mainly used as pharmaceuticals, nutraceuticals, cosmetics and food supplements even as traditional source of medicine (Sharma et aI., 2010). Plant derived product have been used for medical purposes for centuries because it is more effective to treat infectious diseases especially for human such as Neolamarckia cadamba and other medical plant.

Traditional plants have been used before the synthetic antibiotics are introduced in modem medical. In these centuries, the diseases that infected by multi-resistant microbe or antibiotic-resistant microorganism especially from pathogenic fungal strain has increased in the world community. In the past few decades, a worldwide increase in the incidence of fungal infections has been observed as well as a rise in the resistance of some species of fungus to different fungicidal used in medicinal practice (Abadet aI., 2007). Therefore, the investigation for medical plants with antifungal activity and medical values has gained importance in recent years due to a growing worldwide concern about the diseases caused by these pathogenic microorganisms. This is necessary to discover new antimicrobial drug compound among the medical plants in order to overcome the worldwide multi-resistant microorganism.

Neolamarckia cadamba is a broad umbrella-shaped crown that belongs to the Rubiaceae family has been used as traditional medical treatment. Neolamarckia cadamba commonly known as white kelampayan plant in Malaysia is also named as Anthocepha/us chinensis auct., A. cadamba (Roxb.) Miq.. A. indicus A. Rich.. A morindaefolius Korth (Joker, 2000). In this white kelampayan plant, various parts can be used as medical uses and wound healing which are commonly in leaves and bark. According to Dubey et al. (20 II), leaf of N cadamba are slightly aromatic with unpleasant taste but the decoction of leaves is used as gargle in aphthae or stomatitis and in the treatment ofulcers, wound, fever and metorrhea.

Neolamarckia cadamba is one of traditional medical plant that has been mentioned in many Indian medical literatures for the treatment of human disease caused by pathogenio viruses, bacteria and fungi such as diabetes, menorrhagia, anemia, uterine complaints also blood and skin disease (Sher, 2009). This plant contains higher bioactive compound that have a role to maintain human health from various diseases. The bioactivity studies on this N cadamba plant showed its antimicrobial, antioxidant, and wound healing properties. It is also known to possess antimalarial, antihepatotoxic, analgesic hepatoprotective, anti-flammatory, antipryretic, antihelmintic, diuretic, laxative and antidiabetic activities (Zayed et al .. 2014). Thus, the bioactive compound has been shown to possess as a plant ofphytopharmaceutical importance which its leaves can be used as antimicrobial activity (Zayed et al.. 2014).

Antifungal of plant are effective in the treatment of infectious disease and reduce many side-effects that caused by pathogenic fungi since it more environmentally compatible by nature. Therefore, in the presence investigation, the use of fungal growth inhibition to different concentration of plant extracts could help to discover the potential of the antifungal activity on locally Neolamarckia cadamba plant. These techniques are inexpensive and can be perfonned in laboratories with bacterial or fungicidal culture capabilities because it used of crude plant extract against fungal pathogen may inhibit the

development of resistance in the pathogen population due to different antifungal compounds contained in plant extract (Mahlo et al., 2010). Thus, fungal growth of inhibition has an ability to discover the antifungal activity of Neolamarckia cadamba extraction.

Therefore the objectives of this study are:

1. To extract crude leaf extract of Neolamarckia cadamba by using aqueous and ethanol extraction.

2. To detennine the presence of antifungal properties in different concentration of crude extract by efficiency on fungal growth of experimental fungi in poisoned food technique.

2.0 LITERATURE REVIEW

2.1 Characteristic of *Neolamarckia cadamba*

Neolamarclda cadamba is known as white kelampayan is a large tree with 45 m tall without branches more than 25 m which is in medium sizes and evergreen tree which can grows best on deep, moist and warm type of evergreen and deciduous forest (Ramesh, 2011). This plant is the natural tree that is native to Asia and Southeast Asia including Malaysia. It has lightweight hardwood with poor durability that can be used for pulp, producing low- and medium-quality paper instead of light construction work such as crates, furniture, toys, hardboard and etc. The wood is also used as fuel. According to Krisnawati et al.(2011), the bark is grey, smooth and very light in young tree but rough and longitudinally in old tree while the leaves are dark glossy green above paler mid rib and lateral nerves, opposite, simple sessile to petiole around 2.5-6.3 cm long, ovate to elliptical around 15-50 cm long by 8-25 cm broad which it have larger leaves in young tree.

Figure 2.1: Neolamarckia cadamba leaf

Source: https://www.amazon.com/Neolamarckia-cadamba-FLOWER-Exotic-Seeds/dp/B07DLPJTXV

Neolamarckia cadamba or white kelampayan tree in Malaysia refers to the species of Rubiaceae family which has been widely used in many Indian medical literatures. Throughout human history, people have relied on natural products and plants in particular to promote and maintain good health and to fight sickness, pain and disease, but the traditional knowledge related to medicinal properties and uses of plants and other natural product has been eroded. Then, recent studies also have proved that N. cadamba has its own beneficial in the treatment of various ailments like diabetes mellitus diarrhea, fever, inflammation, haemoptysis, cough, vomiting, wound, ulcers, debility and the decoction of leaves is recommended as a gargle in cases of stomatitis (Dubey et al., 2011). According to Palshikar et al. (2013), the wound and ulcers are dressed with its leaves slightly warmed to alleviate the pain, swelling and for cleansing and better healing wounds.

Apart from that, Neolamarckia cadamba has been commonly used as medical literatures because it has wide range of biological activities such as antioxidant, anesthetic, antiseptic, antidiabetic, antimicrobial especially as antifungal activity (Dubey et al., 2011) and hypocholesterolemic which are contains various phytochemical compound. One of those is the studies from Divyakant et al.which the leaf extract of Neolamarckia cadamba given more antifungal activity than the bark extracts with aqueous and alcoholic extracts (ethanol) against different fungi, Aspergillus

This white kelampayan leaves (Figure 2.1) possess various potent bioactive compound that identified various biological activities and recommended as a plant of phytopharmceutical importance (Zayed et al., 2014). On the other hand, various chemical composition have been identified from N. cadamba which their leaves extract revealed the presence of various secondary metabolites and these include glycosides, indole

alkaloids, sapogenins, tannins, phenolic, steroids, and flavonoids (Usman et aI., 2012; Madhu et al., 2012). This plant also contains hentriacontanol and beta-sitosterol as

chemical constituent (Ramesh, 2011).

2.2 Extraction of Neolamarckia cadamba Solvent

According to Chandrashekar and Prasanna (2009), the research is based on antimicrobial activities of Anthocephalus cadamba which also known as Neolamarckia cadamba that used different solvent which are petroleum ether, chloroform and acetone. Chloroform and acetone are effective solvent used to extract antimicrobial compound of N. cadamba. This might due to chloroform and acetone has potential to inhibit the growth of fungi and concentration used is more condense that give more effective result. However, most of the studies of extraction of crude extract from medical plant using ethanol or methanol as solvent. The reason is crude extract of the plant with ethanol or methanol may have mixture of high bioactive compound against pathogenic fungi based on concentration of the extract (Nagappan, 2012). Moreover, the solvent extraction that used in other research about antifungal activity of N. cadamba leaf extract is aqueous and also ethanol solvents (Divyakant et al., 2011). It shows that crude extract with ethanol solvent shows more antifungal activity than aqueous solvent. Thus, the efficiency of ethanol solvent in antifungal activity is much better than other solvent.

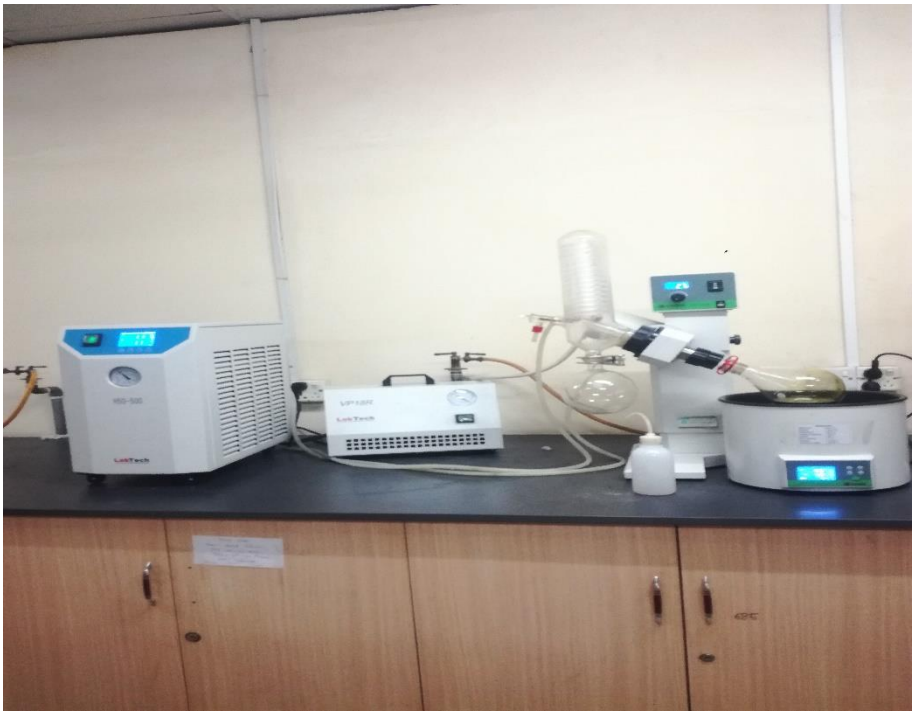

Figure 2.2: Extraction of Neolamarckia *cadamba* Solvent

2.3 Screening of Antifungal Activity

According to Sen and Batra (2012), Minimum Inhibitory Concentration (MIC) is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. The concentration is based on the measurement of the diameters of inhibition growth of each fungus. One of the methods used to evaluate the antifungal properties in plant extract is poisoned food technique. This technique involves the cultivation of the test organism on a medium containing the test chemical and then measuring its growth (Sinclair, 1995) and relevant for antifungal activity in some particular plant extract. The antifungal activity was determined after incubation using measurements of diameter agar disk of the test fungus that seed m center of the poisoned medium.

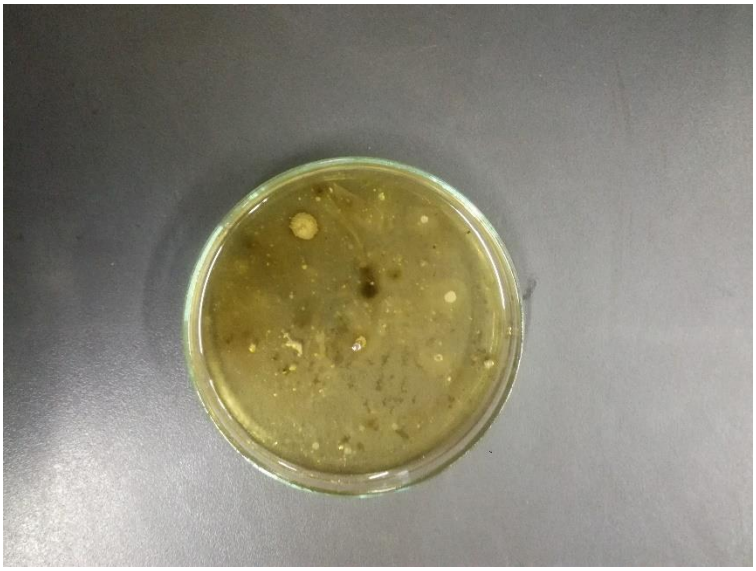

FIGURE 2.3 : Agar Disk

2.4 Selection of Fungi Species

Fungi is one of the microorganism can cause disease in human, plants and animals instead of bacteria and viruses. Mostly, fungal disease are often caused by some fungi that are common in the environment which are live outdoors in soil and on plants and trees as well as on indoor surfaces and on human skin that can harmful to health (CDC, 2014). However, fungi also have important used to human society that provides fundamental products including foods, medicine and enzymes important to industry (Boundless, 2015). Therefore, there are Aspergillus flavus, Aspergillus niger, and Fusarium solani that can use for antifungal activity evaluation

Aspergillus jlavus is pathogenic fungi that cause of human invasive aspergillosis and also one ofAspergillus species can infect the insect. Other than that, this fungus is an opportunistic pathogen of crops which is the major production of aflatoxin as a secondary metabolite in the seeds of a number of crops both before and after harvest (Klinch, 2007). Aspergillus flavus causes a broad spectrum of disease in human, ranging from primary route in inhalation of fungal spores. However, particularly common clinical syndromes associated with A. flavus include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis following trauma and hypersensitivity reactions to invasive infections associated with angioinvasion which have after inoculation (Hedayati et al., 2007).

Aspergillus niger is an important industrial fungus that is widely used for the production of enzymes and secondary metabolites, such as citric acid, but also as a host to produce heterologous proteins. It also has been used to study fungal protein secretion, proteolysis, cell wall biosynthesis, cell morphology and polarity, degradation of plant (cell wall) polysaccharides, central carbon metabolism and nutrient transport, both genetically and biochemically (Gupta et al., 2012). A. niger also a saprophytic and

filamentous fungus found in soil, forage, organic debris and food product causing damage in plants (Avasthi et al., 2010).

Fusarium solani is a phytopathogenic fungus and important causal agent of several crop diseases such as root and fruit rot of Cucurbita spp., root and stem rot of pea, sudden death syndrome of soybean, foot rot of bean and dry rot of potato (Sarah, 2010). However, Fusarium so/ani also infrequent causing human infections such as keratitis which is the most frequent entity, endophtalmitis, onychomycosis, cutaneous and subcutaneous infections, arthritis and disseminated infections. Thus, F. so/ani one of the fungi that can used in study of antimicrobial activity which are there not much infonnation in this study.

3.0 MATERIALS AND METHODS

In Figure 3.0 below show the review process to evaluate antifungal activity of

Neolamarckia cadamba leaf extract that were conducted.
Collection of plant sample
Extraction of plant sample by aqueous and ethanol extraction
Preparation fungal culture and disc inoculum
Finally record and analyze the data

Figure 3.0: The table in methodology of antifungal activity from N cadamba leaf extract.

3.1 Plant Material

The fresh and healthy leaves of the plant Neolamarckia cadamba were collected. The plant samples were washed thoroughly 4-5 times with running tap water. Then, these plants were immersed in distilled water and ethanol to remove any unnecessary particles. Next, the cleaned plant leaves were cut and dried in shaded area for 6-12 days at room temperature (Zayed et al., 2014). Then, the dried leaves were ground into fine powder using mechanical grinder or a blander. The powders were stored in air-tight bottle at 11 room temperature until extraction process. In this experiment, leaves extract were prepared in two types of extraction which are aqueous and ethanol extraction.

3.2 Preparation of Aqueous Extracts

The 10 gram of N. cadamba leaves powder was weighted and macerated with 100 mL sterile distilled water in 250mL capacity of conical flask. The solutions were dissolved or extracted for 72 hours at room temperature in continuous shaker at 130 rpm/min (Obeidat et al., 2012). The macerate was filtered through Buckner funnel and sterile filter paper. Then, extract was preserved aseptically in sterile air-tight bottle at refrigerator condition until further use. The obtained extract served as the crude extract (100 mg/ml). From the stock solution of crude extract, the concentration of 5, 15, 25 and 50 mg/mL extract were prepared.The plants that showed antifungal activity were only selected for further work in solvent extraction (Mohana and Ravessha, 2007).

3.3 Preparation of Alcoholic Extracts

The concentration of 100 mg/ml of ethanol extract was prepared for screenmg of antifungal activity of N cadamba leaves powder. An exact quantity of 10 gram of N cmlmnba leaves powder were weighted and macerated with 100 mL of 80% ethanol in 2SOmL capacity of conical flask. These solutions were dissolved or extracted for 72 hours at room temperature in continuous shaker at 130 rpm/min (Obeidat et aI., 2012). At e end of extraction, ethanol extract were filtered using Buckner funnel and sterile filter paper. The extracts were covered with aluminium foil for evaporated to dryness as to centrate the extract by using 45-50°C oven for 24 hours. After complete solvent evaporation these solvent were weighed and dissolved in 5% dimethylsulfoxide (DMSO) to obtain 100 mg/ml final concentration of crude extract. From the stock lution of crude extract, the concentration of 5, 15, 25 and 50 mg/mL extract were prepared. Then, the extracts were preserved aseptically in sterile air-tight bottles at 4°C

until further use.

3.4 Preparation of Fungal Inoculum Disc

strains Aspergillus flavus, Aspergillus niger, and Fusarium solani were

ected to be experimented for antifungal activity by using different solvent extracts of

this plant. The media used was Potato Dextrose Agar (PDA). The fungal stock cultures

have been isolated for subcultured and incubated for 24 hours at 28°C on PDA medium.

This stock culture have maintained at 4°C on PDA media in refrigerator. The selected fungi were subcultures using PDA medium from stock culture. On the seventh day of incubation, the test fungi were used for the preparation of inoculum disc of 0.5cm in diameter.

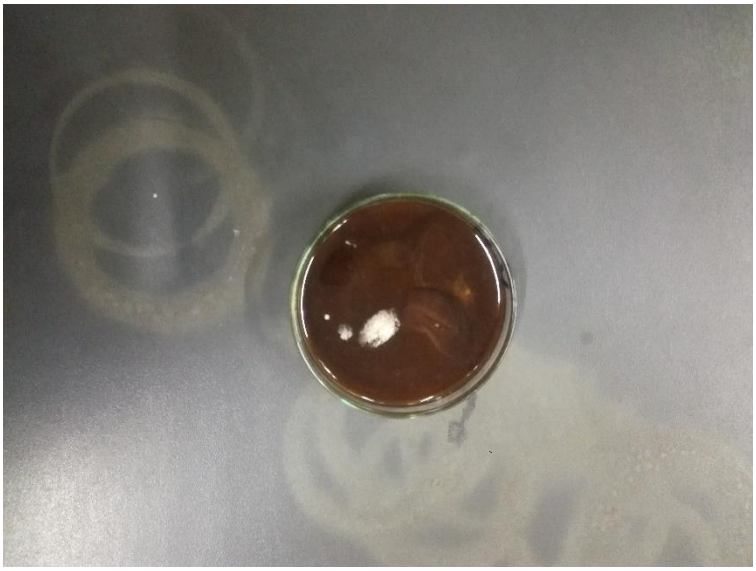

Figure 3.4 : Preparation of Fungal Inoculum Disc

3.5 Antifungal Activity Assay

Determination of fungal inhibition by Poisoned Food Technique

antifungal activity of aqueous and ethanol extracts of N. cadamba plant were

uated by using poisoned food technique to detennine the inhibition of fungal growth

each tested fungi. This method used was based on Kiat and Chiang (2013) with some

For sample treatment, 100 JlI of each leaf extract with different

conceD'trations (5, 15, 25, 50, and 100 mg/ml) was poured into each petri dish at the central and spread over solidified PDA medium with sterile glass spreader and kept in

temperature for absorption of extract in the medium while PDA medium without

tract was served as control. Then, a disk of O.5cm culture of the test fungi was

Placed at the center of both petri dish PDA medium (control and sample treatment) and at dark room at room temperature for 5 days.

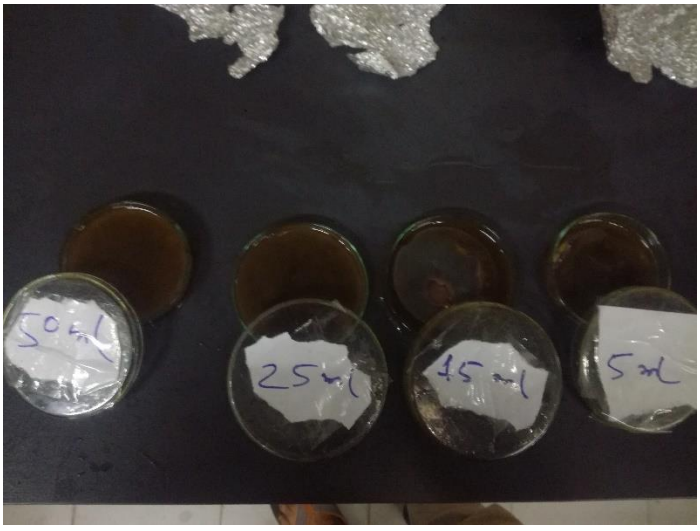

Figure 3.5 : Antifungal Activity Assay

However, the efficiency of each aqueous and ethanol of white kelampayan leaf ..-Inhl!t were evaluated by measuring fungal diameter fungal growth (mm) using ruler on nl and 5th day of interval and in Figure 3.2 illustrates the overview how the measurement was taken. The antifungal activity in terms of percentage inhibition of ' growth was calculated over control by using formula below:

$$\% \text{ IFG} = \frac{DC-DT}{DC} * 100$$

Where, %IFG = Percentage inhibition of fungal growth,

DC= Diameter average of fungal growth in control

DT= Diameter average of fungal growth in treatment

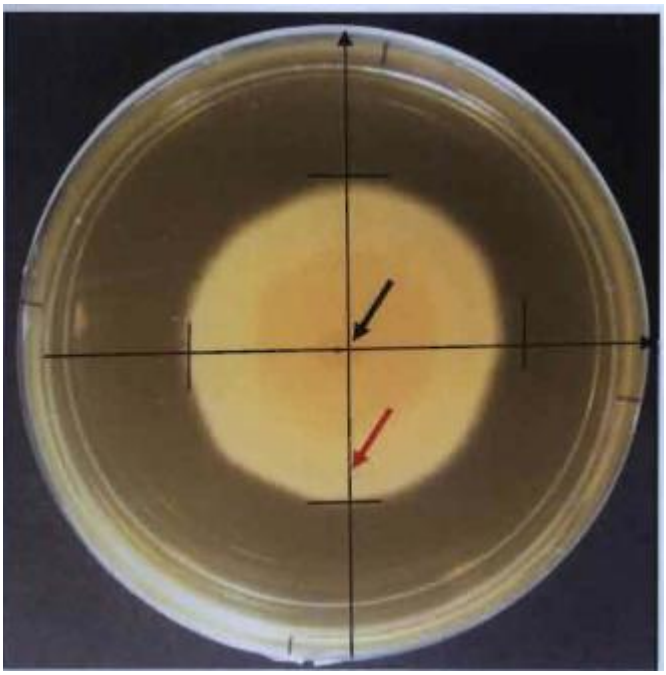

Figure 3.6: PDA medium used for fungus growth inhibition. Black arrow indicates the edge of initial inoculum disc. Red arrow indicates the edge of the fungi diameter growth after inoculation. Each axis corresponds to the four segments used for growth measurement.

4.0 Results and Discussion

Antifungal activity of cadamba leaf was calculated.

%IFG (inhibition of fungal growth) $= \frac{18.9-13.74}{18.9} * 100$

inhibition of fungal growth = 27.3%

5.0 Conclusion & Recommendation

The results of the present study prove that the development of coli was successful in N. cadamba

(Roxb.) The most effective hormonal combinations for Bose bud formation were NA.

5.0 mg / L: BAP 0.5 mg / L and ANS 2.5 mg / L: BAP 3.0 mg / L in MS medium for NPAdambar Leaf-Coli and Internodecli. Although leaf buds and internode extracts seem positive

They have weak antibacterial and antioxidant activities and reveal that secondary metabolism

The cultivar responsible for this national property is produced but a little less. However, natural

The environment places various constraints on the stimulus to produce naturally growing plants

Higher secondary metabolism at higher concentrations. This may be why the leaves and the end

The extracts showed greater antimicrobial and antioxidant activity than that of the coli extract. For example

As suggested in the literature (Indu et al., 23), if calli were subjected to such constraints optimization

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6.0 References

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